

## ORIGINAL ARTICLE

# Complement C3 Variant and the Risk of Age-Related Macular Degeneration

John R.W. Yates, F.R.C.P., Tiina Sepp, Ph.D., Baljinder K. Matharu, M.Sc.,  
Jane C. Khan, F.R.C.Ophth., Deborah A. Thurlby, R.G.N., M.Sc.,  
Humma Shahid, M.R.C.Ophth., David G. Clayton, M.A.,  
Caroline Hayward, Ph.D., Joanne Morgan, B.Sc., Alan F. Wright, Ph.D., F.R.C.P.,  
Ana Maria Armbrrecht, Ph.D., F.R.C.S., Baljean Dhillon, F.R.C.S., F.R.C.Ophth.,  
Ian J. Deary, Ph.D., F.R.C.P.E., Elizabeth Redmond, R.G.N., M.Sc.,  
Alan C. Bird, M.D., F.R.C.S., and Anthony T. Moore, F.R.C.S., F.R.C.Ophth.,  
for the Genetic Factors in AMD Study Group\*

## ABSTRACT

**BACKGROUND**

Age-related macular degeneration is the most common cause of blindness in Western populations. Susceptibility is influenced by age and by genetic and environmental factors. Complement activation is implicated in the pathogenesis.

**METHODS**

We tested for an association between age-related macular degeneration and 13 single-nucleotide polymorphisms (SNPs) spanning the complement genes *C3* and *C5* in case subjects and control subjects from the southeastern region of England. All subjects were examined by an ophthalmologist and had independent grading of fundus photographs to confirm their disease status. To test for replication of the most significant findings, we genotyped a set of Scottish cases and controls.

**RESULTS**

The common functional polymorphism rs2230199 (Arg80Gly) in the *C3* gene, corresponding to the electrophoretic variants C3S (slow) and C3F (fast), was strongly associated with age-related macular degeneration in both the English group (603 cases and 350 controls,  $P=5.9\times 10^{-5}$ ) and the Scottish group (244 cases and 351 controls,  $P=5.0\times 10^{-5}$ ). The odds ratio for age-related macular degeneration in C3 S/F heterozygotes as compared with S/S homozygotes was 1.7 (95% confidence interval [CI], 1.3 to 2.1); for F/F homozygotes, the odds ratio was 2.6 (95% CI, 1.6 to 4.1). The estimated population attributable risk for C3F was 22%.

**CONCLUSIONS**

Complement C3 is important in the pathogenesis of age-related macular degeneration. This finding further underscores the influence of the complement pathway in the pathogenesis of this disease.

From the Cambridge Institute for Medical Research, University of Cambridge, Cambridge (J.R.W.Y., T.S., B.K.M., J.C.K., D.A.T., H.S., D.G.C.); the Medical Research Council Human Genetics Unit, Edinburgh (C.H., J.M., A.F.W.); the Princess Alexandra Eye Pavilion, Edinburgh (A.M.A., B.D.); the University of Edinburgh, Edinburgh (I.J.D.); the Institute of Ophthalmology, University College London (E.R., A.C.B., A.T.M.); and Moorfields Eye Hospital, London (A.C.B., A.T.M.) — all in the United Kingdom. Address reprint requests to Dr. Yates at the Department of Medical Genetics, University of Cambridge, Cambridge Institute for Medical Research, Wellcome Trust/MRC Bldg., Box 139, Addenbrooke's Hospital, Cambridge CB2 0XY, United Kingdom, or at jrwy1@cam.ac.uk.

Drs. Yates and Sepp contributed equally to this article.

\*Other members of the Genetic Factors in Age-Related Macular Degeneration (AMD) Study Group are listed in the Appendix.

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**A**GE-RELATED MACULAR DEGENERATION is the leading cause of visual impairment in the elderly and the most common cause of blindness in Western countries.<sup>1</sup> It affects the macular region of the retina. The macula has a high density of photoreceptors and provides detailed central vision. In the early stages of the disease (referred to as age-related maculopathy), deposits called drusen develop between the retinal pigment epithelium and underlying choroid.<sup>1</sup> Later, the disease is manifested as either extensive atrophy of the retinal pigment epithelium and overlying photoreceptor cells (geographic atrophy) or aberrant choroidal angiogenesis (choroidal neovascularization).<sup>1</sup> Both of these conditions can lead to a loss of central vision. The pathogenesis of age-related macular degeneration is poorly understood. As with other late-onset chronic diseases, susceptibility is influenced by age, ethnic background, and a combination of environmental and genetic factors.<sup>1,2</sup> Smoking status and family history are well-established determinants of risk.<sup>1,2</sup>

Recently, polymorphisms in the genes coding for complement factor H (CFH) and complement factor B (CFB) have been shown to be predictors of risk for age-related macular degeneration.<sup>3-11</sup> Another susceptibility locus has been mapped to chromosome 10q26; the causative variation probably lies in a hypothetical gene called *LOC387715* or in the promoter of the neighboring gene *HTRA1*.<sup>11-14</sup> The population attributable risk associated with variants in *CFH*, *CFB*, and *LOC387715/HTRA1* is at least 50%.<sup>11</sup>

CFH and CFB are key components of the alternative complement pathway. Their involvement in age-related macular degeneration, together with the finding that drusen contain proteins associated with inflammation and immune-mediated processes,<sup>15</sup> supports the hypothesis that inflammation and complement activation influence the pathogenesis of age-related macular degeneration. To test whether variants in other genes encoding proteins in the complement pathway influence susceptibility to age-related macular degeneration, we genotyped single-nucleotide polymorphisms (SNPs) spanning the complement genes *C3* and *C5*, encoding central proteins in the complement cascade, in subjects with age-related macular degeneration and in control subjects.

## METHODS

### CASES AND CONTROLS

We studied three case-control groups, two in England and one in Scotland. English group 1 comprised 446 case subjects with end-stage age-related macular degeneration (geographic atrophy or choroidal neovascularization) and 267 control subjects, who were spouses of the index patients. All subjects were recruited from ophthalmic clinics in eight hospitals in southeastern England from 2002 to 2004.<sup>7</sup> English group 2 comprised 157 case subjects with end-stage age-related macular degeneration and 83 controls (67 spouses and 16 friends of index patients) recruited from 2003 to 2005, the majority from Moorfields Eye Hospital in London and the remainder from southeastern England. All subjects described themselves as "white" rather than "other" on a recruitment questionnaire.

The Scottish group comprised 505 case subjects with age-related maculopathy or end-stage age-related macular degeneration and 351 control subjects. A total of 337 case subjects from the Lothian region were recruited from ophthalmic clinics in Edinburgh and 46 case subjects from hospitals in Dundee and Inverness from 2004 to 2006. Control subjects, who were recruited from the same sources in similar proportions, comprised 32 spouses and 174 subjects who had undergone cataract surgery. Another 122 case subjects and 145 controls came from the 1921 Lothian birth cohort.<sup>16</sup>

Written informed consent was obtained from all subjects. The research protocol was in keeping with the provisions of the Declaration of Helsinki, and approval was obtained from a multicenter research ethics committee and from research ethics committees for each institution. Subjects were examined by an ophthalmologist, and data were collected regarding medical history, lifestyle, and smoking history. Color, stereoscopic fundus photography of the macular region was performed in all subjects. For English subjects, the images were graded at the Reading Centre, Moorfields Eye Hospital, with the use of the International Classification of Age-Related Maculopathy and Macular Degeneration.<sup>17</sup> For Scottish subjects, a study investigator graded images; for validation, images from 100 case subjects and

controls were independently graded at the Moorfields Reading Centre (kappa statistic, 0.84). Eight prospective English controls with age-related macular degeneration and 60 prospective Scottish controls with age-related maculopathy were reclassified as case subjects. Data on disease status, sex, age, and smoking history of subjects are provided in Table 1.

**GENOTYPING**

We extracted genomic DNA from peripheral-blood leukocytes. We selected SNPs spanning the C3 and C5 genes from the International HapMap Project<sup>18</sup> data (release 19) for the Centre d'Étude du Polymorphisme Humain (CEPH) population (Utah residents with ancestry from northern and western Europe). Criteria for the selection of SNPs were high heterozygosity with a minor allele frequency of at least 10%, tagging of the most common haplotypes, and coverage of the main

blocks of linkage disequilibrium. The C3 SNP rs2230199 — which is predicted to result in a substitution of a glycine residue for arginine at position 80 (Arg80Gly) — generates the “fast” electrophoretic allotype of C3 (called C3F); the alternative allotype is “slow” (C3S).<sup>19,20</sup> We included this SNP in the analysis to provide extra coverage and because of evidence of a functional difference between the two alleles. On the basis of our initial analysis, we included rs1047286 (Pro292Leu), which has a known association with rs2230199.<sup>20,21</sup> Initial genotyping was carried out in English group 1. Markers of interest were genotyped in group 2 when samples became available. Data from the Scottish group were used for replication.

We performed genotyping in English subjects with the use of a single-nucleotide primer extension assay (ABI Prism SNaPshot Multiplex Kit, Applied Biosystems) and a genetic analyzer (ABI

**Table 1. Disease Status, Sex, Age, and Smoking History of Subjects.\***

Variable	English Subjects (Groups 1 and 2)		Scottish Subjects	
	Controls (N=350)	Case Subjects (N=603)	Controls (N=351)	Case Subjects (N=505)
Disease status — no.				
Age-related maculopathy		0		261
Geographic atrophy		143		55
Choroidal neovascularization		369		189
Geographic atrophy and choroidal neovascularization		91		0
Sex — no. (%)				
Male	151 (43)	270 (45)	152 (43)	190 (38)
Female	199 (57)	333 (55)	199 (57)	315 (62)
Mean age — yr†	75.3±7.8	79.4±7.2	78.0±8.5	77.8±9.2
Smoking history — no. (%)				
No. of subjects	347	595	347	499
Never smoked	141 (41)	214 (36)	161 (46)	240 (48)
Current or former smoker	206 (59)	381 (64)	186 (54)	259 (52)
Quantity of cigarettes smoked‡				
0 pack-yr	141 (41)	214 (36)		
0.1–20.0 pack-yr	126 (36)	166 (28)		
20.1–40.0 pack-yr	61 (18)	136 (23)		
>40.0 pack-yr	19 (5)	79 (13)		

\* Plus-minus values are means ±SD.

† For the English subjects, P<0.001 for the comparisons between case and control subjects. Data on the quantity of cigarettes smoked were not collected for the Scottish subjects.

Prism 3100, Applied Biosystems) and in Scottish subjects — for rs2230199 and rs1047286 — with the use of competitive allele-specific polymerase-chain-reaction assays (Taqman SNP Genotyping Assay, Applied Biosystems and KASPar SNP Genotyping System, KBiosciences, respectively). Manufacturers' protocols were followed.

#### STATISTICAL ANALYSIS

We used the chi-square test for comparisons of categorical variables and allele and genotype frequencies and to check for Hardy–Weinberg equilibrium. All *P* values were calculated with two-sided tests, and no correction was made for multiple testing. The Mann–Whitney *U* test was used to compare the ages of case subjects and controls. Logistic-regression analysis was used to investigate interactions between genotype and other variables and to estimate odds ratios and 95% confidence intervals. The covariables of age and smoking history were included in the logistic model if univariate analysis had shown a significant difference. Odds ratios for categorical variables were estimated in relation to a reference category. Data were analyzed with the use of the SPSS statistical software package, version 11.0.

The population attributable risk was calculated from the formula  $100D \div (1 + D)$ , in which *D* was equal to  $P_1(RR_1 - 1) + P_2(RR_2 - 1)$ , where  $P_1$  and  $P_2$  are the frequencies of the at-risk genotypes, and  $RR_1$  and  $RR_2$  their associated relative risks, as compared with the low-risk genotype. For the purposes of estimation, odds ratios were equated to relative risks, since the disease prevalence is low.

## RESULTS

In the initial screening, 12 SNPs spanning *C3* and *C5* (those listed in Table 2, excluding rs1047286) were genotyped in 446 case subjects with late-stage age-related macular degeneration and 267 control subjects (English group 1). No evidence of an association was found with variants in *C5* (Table 2). In *C3*, the expressed SNP rs2230199 showed strong evidence of an association ( $P < 0.001$ ) and was genotyped in an additional 157 case subjects and 83 controls (English group 2). The enlarged sample also provided strong evidence of an association ( $P = 5.9 \times 10^{-5}$ ) (Table 2).

To test for replication of this finding, rs2230199

was genotyped by a different laboratory in 244 case subjects with late-stage age-related macular degeneration, 261 case subjects with age-related maculopathy, and 351 controls (Scottish group). Again, there was a highly significant association between the minor allele and age-related macular degeneration ( $P = 5.0 \times 10^{-5}$ ) (Table 3). International HapMap Project<sup>18</sup> data for the CEPH population showed that rs2230199 had an  $r^2$  value of 0.75 with rs2230203 but a low  $r^2$  value with other *C3* SNPs in our marker panel and with other *C3* SNPs in the HapMap data set. SNP rs2230203 did not show a significant association with age-related macular degeneration in group 1 alone, but there was weak evidence of an association in groups 1 and 2 combined (Table 2).

Because of the known association between the allotypes of rs2230199 and the expressed *C3* SNP rs1047286, the English and Scottish subjects were genotyped for this marker (Tables 2 and 3). The minor allele frequency was significantly higher in case subjects than in controls in both groups, but the association was not as strong as for rs2230199. Stepwise logistic-regression analysis confirmed that rs2230199 is a significantly better predictor of risk for age-related macular degeneration. With this SNP in the model, adding rs1047286 made no contribution ( $P = 0.90$ ). With rs1047286 in the model, adding rs2230199 produced a significant improvement in fit ( $P = 0.02$ ).

Odds ratios for age-related macular degeneration as a function of rs2230199 genotype are given in Table 4. Results for the English and Scottish groups were similar. In the combined data set, with the common CC genotype as the reference, the odds ratio was 1.7 for CG heterozygotes and 2.6 for GG homozygotes. The estimated population attributable risk for this variant was 22%.

Subgroup analysis that was confined to case subjects with only choroidal neovascularization showed a highly significant association in both case–control groups. For case subjects with only geographic atrophy, the association was significant in the English group ( $P = 4.6 \times 10^{-4}$ ) but not in the Scottish group, which had fewer subjects with geographic atrophy. The Scottish group included case subjects with age-related maculopathy, and in this subgroup the association fell just short of significance (Table 3).

Data on other susceptibility loci for age-related macular degeneration were available for English group 1. Results for *CFH* Y402H have been pub-

**Table 2. Genotyping Results for English Subjects.\***

Gene and SNP ID (Location)	Amino Acid Data		Controls		Case Subjects			P Value†		
	AA	Aa	AA	Aa	AA	Aa	aa	Allele	Genotype	
C3	number (percent)		number (percent)		number (percent)					
rs2250656 (6658534 bp, intron 2)	128 (50)	108 (42)	20 (8)	20 (8)	248 (57)	159 (36)	30 (7)	0.25	0.12	0.23
rs2230199 (6658387 bp, exon 3)	223 (64)	109 (32)	14 (4)	14 (4)	303 (51)	242 (41)	45 (8)	0.28	5.9×10 <sup>-5</sup>	2.8×10 <sup>-4</sup>
rs1047286 (6653262 bp, exon 9)	227 (65)	106 (30)	17 (5)	17 (5)	323 (54)	230 (38)	49 (8)	0.27	4.0×10 <sup>-4</sup>	2.3×10 <sup>-3</sup>
rs2230203 (6650782 bp, exon 13)	235 (67)	99 (28)	16 (5)	16 (5)	354 (59)	207 (34)	41 (7)	0.24	0.007	0.03
rs2230204 (6649848 bp, exon 14)	126 (47)	113 (42)	27 (10)	27 (10)	233 (52)	177 (40)	34 (8)	0.28	0.13	0.31
rs2287846 (6636557 bp, intron 22)	97 (37)	121 (47)	42 (16)	42 (16)	164 (38)	208 (48)	64 (15)	0.38	0.74	0.87
rs344542 (6627517 bp, intron 27)	105 (41)	119 (46)	35 (14)	35 (14)	158 (36)	227 (52)	54 (12)	0.38	0.53	0.34
rs2241393 (6625304 bp, intron 29)	94 (36)	122 (47)	45 (17)	45 (17)	183 (42)	196 (45)	57 (13)	0.36	0.06	0.17
rs344550 (6622953 bp, intron 33)	109 (45)	106 (44)	28 (12)	28 (12)	186 (44)	184 (43)	53 (13)	0.34	0.73	0.92
rs2277984 (6619511 bp, intron 36)	68 (27)	126 (50)	60 (24)	60 (24)	118 (28)	201 (47)	108 (25)	0.49	0.89	0.80
C5										
rs7033790 (311109597 bp, intron 8)	137 (55)	100 (40)	13 (5)	13 (5)	233 (59)	140 (35)	24 (6)	0.24	0.53	0.47
rs17611 (31090405 bp, exon 19)	79 (31)	132 (53)	40 (16)	40 (16)	141 (33)	204 (47)	86 (20)	0.44	0.62	0.31
rs7026551 (31054338 bp, intron 31)	160 (63)	82 (33)	10 (4)	10 (4)	269 (63)	138 (33)	17 (4)	0.20	0.98	1.00

\* Because of genotyping failures, data were not available for all subjects. Genotype frequencies are given as percentages of subjects genotyped. The location of single-nucleotide polymorphisms (SNPs) in C3 and C5 is specified by the position on a contig map of chromosomes 19 and 9, respectively, from the National Center for Biotechnology Information's SNP Database, build 127. The numbering of the C3 amino acid sequence excludes the 22 residues of the signal peptide present in precursor C3 (pro-C3). Percentages may not total 100 because of rounding. "A" denotes common allele, "a" minor allele, and MAF minor allele frequency.

† P values were calculated with the chi-square test and are for comparisons of allele and genotype frequencies between case subjects and controls.

**Table 3.** Genotyping Results for Scottish Replication Group.\*

C3 Gene SNP ID	Amino Acid Data	Controls				Case Subjects				P Value†	
		AA	Aa	aa	MAF	AA	Aa	aa	MAF	Allele	Genotype
		number (percent)				number (percent)					
rs2230199	Arg80Gly (slow/fast allotype)	215 (65)	103 (31)	14 (4)	0.20	117 (49)	100 (42)	22 (9)	0.30	5.0×10 <sup>-5</sup>	3.3×10 <sup>-4</sup>
	Age-related macular degeneration	215 (65)	103 (31)	14 (4)	0.20	141 (56)	96 (38)	13 (5)	0.24	0.06	0.12
	Age-related maculopathy	215 (65)	103 (31)	14 (4)	0.20	258 (53)	196 (40)	35 (7)	0.27	5.2×10 <sup>-4</sup>	2.2×10 <sup>-3</sup>
	All subjects	210 (64)	103 (32)	13 (4)	0.20	116 (51)	94 (41)	18 (8)	0.29	7.3×10 <sup>-4</sup>	3.4×10 <sup>-3</sup>
rs1047286	Pro292Leu (HAV4-1 allotype)										
	Age-related macular degeneration										

\* Because of genotyping failures, data were not available for all subjects. Genotype frequencies are given as percentages of subjects genotyped. The numbering of the C3 amino acid sequence excludes the 22 residues of the signal peptide present in precursor C3 (pro-C3). "A" denotes common allele, "a" minor allele, and MAF minor allele frequency.

† P values were calculated with the chi-square test and are for comparisons of allele and genotype frequencies between case subjects and controls.

lished previously and are in agreement with other reports.<sup>7</sup> Odds ratios and population attributable risks for *LOC387715* (rs10490924) and *CFB* (rs641153) are given in Table 5. The results are similar to those of other studies, except that we found a lower odds ratio for rs10490924 homozygotes. When these variables were included in the stepwise logistic model, C3 rs2230199 remained significant, with an odds ratio of 1.4 for CG heterozygotes and 3.3 for GG homozygotes (with CC genotype as the reference), confirming that these susceptibility loci are independent risk factors.

## DISCUSSION

Our study showed a strong association between the complement C3 S/F (Arg80Gly) polymorphism and age-related macular degeneration, with similar findings for geographic atrophy and choroidal neovascularization. The C3F allele frequency is approximately 20% in white populations but lower in other ethnic groups. For age-related maculopathy, the association fell just short of significance, raising the possibility that this polymorphism has less influence on the earlier stages of the disease.

The complement system comprises more than 30 plasma and cell-surface proteins. It mediates the host defense against pathogens and the elimination of immune complexes and apoptotic cells; it also facilitates adaptive immune responses.<sup>22</sup> C3 is the most abundant complement component, synthesized predominantly in the liver but to a lesser extent in other cells and tissues. Significant C3 messenger RNA is detectable in the neural retina, choroid, retinal pigment epithelium, and cultured retinal-pigment-epithelium cells.<sup>15</sup>

Cleavage of C3 into C3a and C3b is the central step in complement activation and can be initiated by the classic antibody-mediated pathway, the lectin pathway, or the alternative complement pathway.<sup>22</sup> C3b attaches to pathogens or other target surfaces and binds factor B, which is then cleaved. The resulting C3bBb complex has C3 convertase activity, which amplifies the response by further cleavage of C3 and leads to the formation of C3b<sub>2</sub>Bb complexes with C5 convertase activity. This brings about cleavage of component C5 and recruitment by C5b of components C6 through C9 to form a large molecular pore on target membranes (the membrane attack complex), resulting in cell lysis.<sup>22</sup>

Drusen contain C3 and its activation products, as well as C5, membrane attack complex, and CFH,<sup>6,15</sup> supporting the hypothesis that local inflammation and activation of the complement cascade contribute to the pathogenesis of age-related macular degeneration. Further support for this hypothesis comes from conclusive evidence that variants in CFH influence susceptibility to age-related macular degeneration.<sup>3-9</sup> CFH is a key regulator of the alternative complement pathway and prevents uncontrolled complement activation. Variants in factor B also appear to influence susceptibility to age-related macular degeneration.<sup>10,11</sup> In mice, activation of complement and formation of the membrane attack complex are essential for the development of laser-induced choroidal neovascularization. Indeed, the finding that choroidal neovascularization cannot be induced by laser coagulation in C3<sup>-/-</sup> mice demonstrates the key role of C3 in this process.<sup>23</sup>

As a result of cleavage of C3 to form C3b, the molecule undergoes conformational changes that expose several binding sites, including the thioester moiety, which is essential for C3b binding to target surfaces.<sup>24</sup> Exposure of this activated acyl-imidazole intermediate requires a substantial relocation of the thioester-containing domain to a position adjacent to the first macroglobulin domain.<sup>24</sup> Arg80 together with Arg72 and Lys82 forms a positively charged patch on the surface of this domain, which, in C3b, is brought into close proximity with the negatively charged carboxyl groups of several amino acids on the surface of the thioester-containing domain (Fig. 1). Substitution of an uncharged glycine for the positively charged Arg80 is predicted to weaken the interaction between these oppositely charged surfaces and could potentially influence thioester activity or other binding interactions of the thioester-containing domain, including a probable C3b/C3d binding site with CFH.<sup>25</sup> It follows that there could well be functional differences between the C3 S/F variants.

Direct experimental evidence of functional differences in vitro between the C3 S/F allotypes is not conclusive. Arvilommi<sup>26</sup> reported that erythrocytes coated with C3F showed greater rosetting with peripheral-blood mononuclear cells than those coated with C3S. Welch et al.<sup>27</sup> studied uptake on sheep erythrocytes, hemolytic activity, conversion to inactive C3b, and capacity to solu-

**Table 4. Complement C3 rs2230199 Genotype (C3 S/F Allotype) and Odds Ratios for Age-Related Macular Degeneration.\***

Group	Odds Ratio (95% CI)	
	CG (C3 S/F Allotype)	GG (C3 F/F Allotype)
English subjects	1.6 (1.2–2.2)	2.4 (1.3–4.4)
Scottish subjects	1.8 (1.2–2.6)	2.9 (1.4–5.9)
Combined groups	1.7 (1.3–2.1)	2.6 (1.6–4.1)

\* Odds ratios are for the comparison with the CC genotype (C3 S/S allotype).

**Table 5. Odds Ratios for Age-Related Macular Degeneration and Population Attributable Risk for Variants at the Susceptibility Loci CFH, CFB, and LOC387715 in English Group 1.\***

Locus (Variant)	Odds Ratio (95% CI)		Population Attributable Risk
	Aa	aa	%
CFH (Y402H) <sup>7</sup>	3.1 (2.0–4.6)	6.3 (3.8–10.4)	63
CFB (rs641153)	0.5 (0.3–0.8)	0.2 (0.02–1.8)	77
LOC387715 (rs10490924)	2.4 (1.6–3.6)	2.4 (1.5–3.8)	43

\* Odds ratios are for the comparison with the AA genotype. “A” denotes common allele, and “a” minor allele.

bilize preformed immune complexes. The only significant difference was that C3F had lower activity than C3S in a hemolytic assay using sensitized sheep erythrocytes as a result of a small difference in cell-surface binding. Bartók and Walport<sup>28</sup> found no differences between binding of C3S and C3F and the major complement receptor types 1, 2, and 3.

On the other hand, there is compelling indirect evidence of a functional difference between C3S and C3F. A recent study has shown that the C3 S/F genotype is an important determinant of the long-term outcome of renal transplantation.<sup>29</sup> In recipients who were C3S homozygotes, graft survival was substantially prolonged and renal function significantly better with C3 F/F and C3 F/S donor kidneys than with C3 S/S kidneys.

Several associations of disease with C3F have been reported, including IgA nephropathy,<sup>30</sup> systemic vasculitis,<sup>31</sup> partial lipodystrophy, and membranoproliferative glomerulonephritis type II (MPGNII).<sup>32,33</sup> The association with MPGNII is particularly relevant. This is a rare disease characterized by complement-containing dense deposits in the glomerular basement membrane of the



**Figure 1. Structure of Complement C3b, Showing the Location of Arg80.**

Ribbon representation of the structure of complement C3b, as proposed by Janssen et al.,<sup>24</sup> shows the interface region between the macroglobulin 1 domain (MG1) (light blue) and the thioester-containing domain (orange). The residues participating in the formation of the thioester bond are shown in yellow. Arg80 (red) is located in MG1, adjacent to two other positively charged amino acids, Arg72 and Lys82 (purple). These residues are approximately 4 Å from the negatively charged amino acids Asp1007, Glu1008, Glu1010, and Glu1013 (dark blue) in the thioester-containing domain. The first three of these residues contribute to a probable C3b/C3d binding site with complement factor H.<sup>25</sup> Arg80 may also have interactions with negatively charged residues (not shown) in the complement C1r/C1s–Uegf–Bmp1-containing domain, adjacent to the thioester-containing domain.

kidney.<sup>34</sup> The condition is caused by uncontrolled activation of the alternative complement pathway. In the majority of patients, the condition is associ-

ated with serum C3 nephritic factor,<sup>34</sup> an auto-antibody directed against the C3bBb complex, but rare cases associated with mutations in CFH have been reported.<sup>35</sup> A similar form of glomerulonephritis develops in CFH-deficient pigs<sup>36</sup> and CFH knockout mice.<sup>37</sup> The interface of the capillary tuft, the glomerular basement membrane, and the glomerular epithelial cells in the kidney is similar in structure to the interface involving choriocapillaris, Bruch's membrane, and retinal pigment epithelium in the eye, and macular drusen similar to those in age-related macular degeneration develop in patients with MPGNII, but at a much younger age.<sup>38</sup> These lesions are structurally and compositionally identical to those in patients with age-related macular degeneration and show immunoreactivity to complement C5 and C5b-9 complexes.<sup>39</sup> Drusen have also been reported in patients with partial lipodystrophy.<sup>40</sup> The association of MPGNII and partial lipodystrophy with C3F fits well with our current findings.

In summary, our study shows a strong association between the C3F variant and age-related macular degeneration, and there is evidence of functional differences between the C3 S/F allotypes. It follows that C3F is likely to have a causal role in the disorder. The estimated population attributable risk in the white population is 22%. These findings add to our growing understanding of the genetics of age-related macular degeneration and provide conclusive evidence that the complement pathway has a key role in the pathogenesis of this common and debilitating condition.

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## APPENDIX

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